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(54) Title: INDIGOID BISINDOLE DERIVATIVES			
(57) Abstract The present invention relates to novel indigoid binsindole derivatives which can be used for the manufacture of a medicament for the treatment of solid cancers.			

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Title: "Indigoid bisindole derivatives"

Description

The present invention relates to novel indigoid bisindole derivatives which can be used for the manufacture of a medicament for the treatment of solid cancers.

Indigoid bisindoles comprise a spectrum of natural dye stuffs. Many of these can be obtained from plants. Accordingly, indirubin, indigo and isoindigo are natural products which can be obtained from different plants: namely, *Baphicacanthus cusia* (Acanthaceae), *Indigofera suffruticosa* (Fabaceae), *Isatis indigotica* (Brassicaceae) and others. Indican, a glycoside which is found in plants, gives glucose and 3-hydroxyindole due to acidic or enzymatic hydrolysis. 3-Hydroxyindole is converted by air-oxidation into indigo and its isomers. Indigo naturalis (Chinese: Quing Dai) is the natural blue dye obtained from plant material, e.g. *Isatis indigotica* (Brassicaceae). Indirubin, an isomer of indigo, can be found in Indigo naturalis in an amount of up to 60% (Falbe J. & Regitz M., *R̄ mpp Chemie Lexikon* (1992), 9. Aufl., Stuttgart, Georg Thieme Verlag). It occurs also in *Isatis tinctoria* in an amount of up to 5% which is indigenous to Central Europe (Gelius R., *Z. Chem.*, 20, (1980), 340-341). Derivatives of indirubin are known for a long time as dyes of low persistence.

Indigo naturalis is reported to be used in traditional Chinese medicine as a haemostatic, anti-pyretic, anti-inflammatory and sedative agent in the treatment of bacterial and viral infections. Antileukemic effects of Indigo naturalis have also been reported, with indirubin being the effective principle (Ji X. et al., *Acta Pharm. Sin.*, 16, (1981), 146-148; Gan W. J. et al., *J. Hematol.*, 6, (1985), 611-613). In spite of its anti-leukaemic activity, however, indirubin dissolves only poorly in water and is therefore not readily resorbed. Recently, the antileukemic activity of some better soluble indirubin derivatives has been reported (Ch. Li et al., *Bull. Chem. Soc. Jpn.* 69, 1621-1627, (1996)).

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However, indigoid bisindole or its derivatives have never been investigated with respect to solid tumors, in particular human solid tumors, and furthermore, the problem of the poor solubility resulting in a poor resorption has not been sufficiently solved yet.

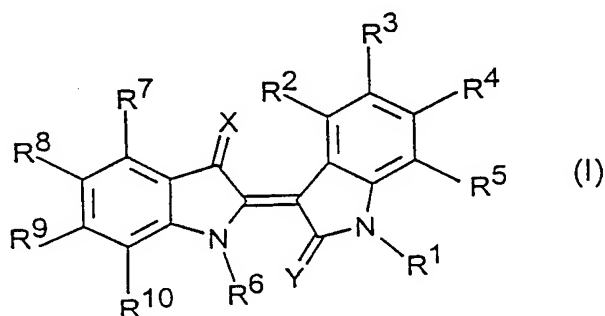
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Thus, the technical problem underlying the present invention is to provide new active substances which can be used in the treatment of human solid tumors and metastases thereof. Furthermore, the resorbability of said substances should be improved in order to improve their *in vivo* anti-tumor activity.

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The solution to the above technical problem is achieved by the embodiments characterized in the claims.

In particular, the present invention relates to cell membrane penetrating indigoid bisindole derivatives selected from indigo derivatives, isoindigo derivatives and indirubin derivatives wherein the indigoid bisindole derivatives are compounds having the general formula (I)



wherein:

20 X and Y are the same or different and represent an oxygen atom; a sulphur atom; a selenium atom; a tellurium atom; a group N-A-B-R¹⁴ in which A represents a single bond or an oxygen atom, -NH- or -NH-CO-, B represents a single bond or a group [(CD₂)_nZ]_m wherein D has the same meaning as R¹⁴ (see below) and Z is an oxygen atom or -NH-, n is 0 or an integer and m is an integer; and the group R¹⁴

25 represents a hydrogen atom, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can carry one or more hydroxy and/or amino groups and can be substituted by one or more carboxyl groups and/or phosphoryl groups, a substituted or unsubstituted aryl group which can comprise one or more

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heteroatoms, an aralkyl group, an acyl group, a glycoside selected from monosaccharides, disaccharides or oligosaccharides, or a group selected from the group consisting of sugars, amino acids, peptides or steroid hormones; or a hydrazone group $N-NR^{15}R^{16}$, wherein R^{15} and R^{16} can be the same or different and
5 represent a hydrogen atom, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can be substituted by one or more carboxyl groups and/or phosphoryl groups,, a substituted or unsubstituted aryl group which can comprise one or more heteroatoms, an aralkyl group, an acyl group, or a glycoside selected from monosaccharides, disaccharides or oligosaccharides, or a
10 group selected from the group consisting of sugars, amino acids, peptides or steroid hormones;

R^2 , R^3 , R^4 , R^5 , R^7 , R^8 , R^9 and R^{10} can be the same or different and represent a hydrogen atom; a halogen atom; a hydroxy group; a nitroso group; a nitro group; an aryloxy group; an alkoxy group; a straight-chain or branched-chain alkyl group
15 having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups; a substituted or unsubstituted aryl group which can comprise one or more heteroatoms; a cycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatoms; an aralkyl group; a trifluoromethyl group; a -COM group; a -COOM group; a -CH₂COOM group, wherein M is
20 hydrogen, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, or an aryl group which can comprise one or more heteroatoms and can be substituted with one or more halogen atoms, one or more alkyl groups or one or more alkoxy groups; a -NR¹¹R¹² group, wherein R^{11} and R^{12} can be the same or
25 different and represent a hydrogen atom, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, a substituted or unsubstituted aryl group which can comprise one or more heteroatoms, or an acyl group, or R^{11} and R^{12} form together a ring having 2 to 6, optionally substituted, CH₂ groups; a benzyl group, wherein
30 the benzene nucleus can comprise one or more heteroatoms; a hydroxylamino group; a phosphate group; a phosphonate group; a sulfate group; a sulfonamide group, wherein the nitrogen atom can be independently substituted by a hydrogen atom, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, a

- substituted or unsubstituted aryl group or wherein the nitrogen atom is part of a cycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatoms; an azo group $\text{N}=\text{N}-\text{R}^{13}$, in which R^{13} represents an aromatic system which can be substituted by one or more carboxyl groups and/or phosphoryl groups; or a O-glycoside or a N-glycoside, wherein the glycoside is selected from monosaccharides, disaccharides or oligosaccharides; or a group selected from the group consisting of sugars, amino acids, peptides or steroid hormones; or R^1 and R^5 , and R^6 and R^{10} , respectively, form independently from each other a ring together having 1 to 4, optionally substituted, CH_2 groups;
- the groups R^1 and R^6 are the same or different and represent a hydrogen atom; a halogen atom; a hydroxy group; a methylenehydroxy group; a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms; a cycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatoms; a substituted or unsubstituted aryl group which can comprise one or more heteroatoms; a mono-, di- or trialkylsilyl group having 1 to 6 carbon atoms independently of each other in each instance in the straight-chain or branched-chain alkyl group; a mono-, di- or triarylsilyl group with substituted or unsubstituted aryl groups independently of each other in each instance; an aralkyl group; a trifluoromethyl group; a $-\text{COM}$ group; a $-\text{COOM}$ group; a $-\text{CH}_2\text{COOM}$ group, wherein M is hydrogen, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, or an aryl group which can comprise one or more heteroatoms and can be substituted with one or more halogen atoms, one or more alkyl groups or one or more alkoxy groups; a $-\text{NR}^{17}\text{R}^{18}$ group, wherein R^{17} and R^{18} can be the same or different and represent a hydrogen atom, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, a substituted or unsubstituted aryl group which can comprise one or more heteroatoms, or an acyl group; a methyleneamino group $-\text{CH}_2-\text{NR}^{17}\text{R}^{18}$, wherein R^{17} and R^{18} have the above definitions; a benzyl group, wherein the benzene nucleus can comprise one or more heteroatoms; a methylenecycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatoms; a physiological amino acid residue bound to the nitrogen as an amide; an O-glycoside or a N-glycoside, wherein the glycoside is selected from monosaccharides, disaccharides or oligosaccharides; or a group selected from the

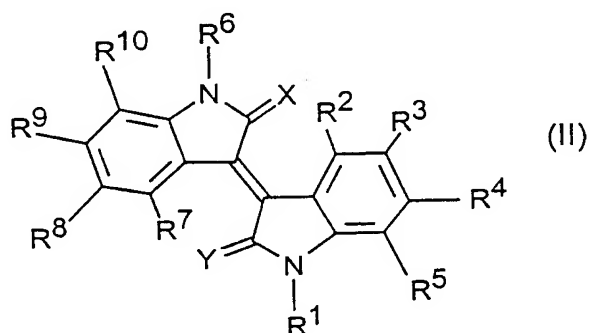
group consisting of sugars, amino acids, peptides or steroid hormones; or a methylene sulfonate group.

The above mentioned sugars can e.g. be connected to the indigoid bisindole derivative as a N-glycoside or an O-glycoside, such as a β -D-glycoside, and the glycoside is preferably selected from mono-, di- and oligosaccharides.

The above mentioned peptide may be an oligo- or polypeptide and may e.g. be a NH-CO-peptide sequence or a NH-CO-peptide-polymer sequence employed as a typical substrate for tumor associated proteases (e.g. plasmin, cathepsin and collagenases). Such peptide sequences can be e.g. D-Ala-Phen-Lys, D-Val-Leu-Lys or Gly-Phen-Leu-Gly. The number of amino acids within the peptide sequence is preferably 1 to 6 amino acids. In the NH-CO-peptide-polymer sequence the polymer is not limited and e.g. hydroxypropyl methacrylamid copolymers can be used.

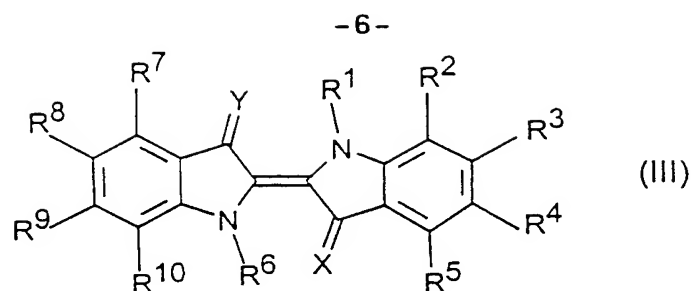
The above mentioned steroid hormone can e.g. be selected from glucocorticoids or sex hormones such as androgens, oestrogens and gestagens.

The isoindigo derivative is preferably a compound having the general formula (II)



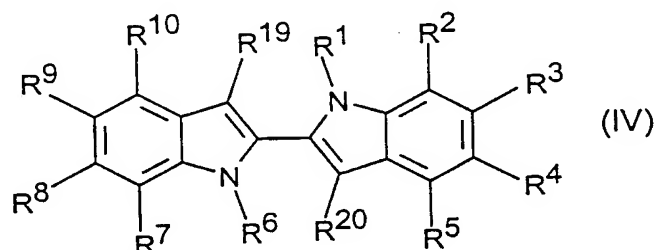
wherein R¹ to R¹⁰ and X and Y have the meanings as defined above.

Preferably, the indigo derivative is a compound having the general formula (III)



wherein R^1 to R^{10} and X and Y have the meanings as defined above.

According to another embodiment of the present invention, the indigo compound
5 may further be a compound having the general formula (IV):



wherein R^1 to R^{10} have the meaning as defined above and R^{19} and R^{20} which may
be the same or different have the same meaning as defined for e.g. R^2 above.

10

In the above indigoid bisindole derivatives having the general formulae (I), (II) and
(III), one or more ring atoms of the benzene nuclei may be replaced by nitrogen
atoms. Furthermore, the indigoid bisindole derivatives having the general formulae
(I), (II) and (III) may according to one embodiment of the present invention be
15 bound to a polyethyleneglycolester or a polyethyleneglycol-ether.

In the above formulae (I), (II) and (III), Y preferably represents an oxygen atom
and R^1 preferably represents a hydrogen atom.

20 The indigoid bisindole derivatives according to the present invention may also be
chemically coupled to masking agents as described in German patent application
DE-A-38 27 488 which function to carry the anti-tumor active substances to the
tumor.

In the following, the indigoid bisindole derivatives selected from indigo, isoindigo and indirubin derivatives according to the present invention are also addressed to as "anti-tumor active compounds according to the present invention".

- 5 The anti-tumor active compounds according to the present invention can be used for the manufacture of a medicament for the treatment of human solid tumors and metastases thereof. The term "human solid tumors" according to the present invention preferably includes carcinomas, melanomas, adenomas, sarcomas, lymphomas, neuroblastomas, teratomas and astrocytomas. Specific examples are
- 10 mammary carcinoma, large-cell lung carcinoma, small-cell lung carcinoma, lung adenocarcinoma, colon carcinoma, bladder carcinoma, ovarian carcinoma, pancreatic carcinoma, renal carcinoma, prostatic carcinoma, bronchial carcinoma, laryngeal carcinoma and the like.
- 15 One general problem in the field of pharmacology is the formulation of pharmaceutically active substances in pharmaceutical compositions which can be applied to a human body. Since most physiological fluids are water-based, the pharmaceutically active substances should be soluble in water and/or a water mixable solvent wherein the latter of course has to be physiologically acceptable
- 20 in small concentrations, such as ethanol. Furthermore, pharmaceutically active substances which are taken orally have to be resorbed into surface of the human body including the gastrointestinal mucous membrane or, in case of an application via syringe, e.g. intraperitoneal or intravasal, have to be resorbed through the cellular membranes of the of destination cells, specifically, into the
- 25 tumor cells.

According to the present invention it has been found that in case of the indigoid bisindole derivatives according to the present invention, a good solubility is not the only prerequisite guaranteeing a good anti-tumor activity *in vivo* as it will become

30 apparent by the below Examples. An important factor for the anti-tumor activity of indigoid bisindole derivatives is their ability to penetrate the cellular membranes of the tumor cells. Cellular membranes are composed of lipid bilayers, i.e. compose a rather non-polar medium. Therefore, substitution with very polar groups on the one hand improves the water solubility of a compound but on the other hand

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hinders or even prohibits the resorption of anti-tumor active substances into a tumor cell. Thus, anti-tumor active substances which show good anti-tumor activities under certain *in vitro* conditions, have to be rejected because of not showing any activity when tested using intact cells or *in vivo*.

5

Therefore, the indigoid bisindole derivatives according to the present invention are cell membrane penetrating indigoid bisindoles. According to the present invention the terms "cell membrane penetrating" and "cell resorbable" mean the ability of the indigoid bisindole derivatives to be taken up by the tumor cell through the cellular

10

Therefore, according to a preferred embodiment of the present invention, the indigoid bisindole derivatives according to the present invention are selected from substituted indirubine derivatives, substituted isoindigo derivatives and substituted

15 indigo derivatives, i.e. the groups R^2 to R^5 and R^7 to R^{10} in above formulas (I), (II) and (III) do not all represent hydrogen atoms simultaneously. In case of the indirubine derivatives according to above formula (I), the groups R^2 to R^5 and R^7 to R^{10} may all represent hydrogen atoms if the group X represents a group $N-A-B-R^{14}$ wherein A, B and R^{14} have the above defined meaning. Even more preferably, the

20 indirubine derivatives, isoindigo derivatives and indigo derivatives are not substituted with easily dissociating, very polar groups, such as a non-substituted sulfonate group SO_3H .

25

The present invention further relates to a pharmaceutical formulation comprising at least one of the indigoid bisindol derivatives according to the present invention in a pharmaceutically active amount.

30

In the pharmaceutical formulations according to the present invention, the indigoid bisindole derivatives can also be employed in the form of their physiologically acceptable salts. The above identified indigoid bisindole derivatives of the present invention can be formulated into pharmaceutical compositions which contain optionally a pharmaceutically acceptable carrier and/or diluent. Said pharmaceutical compositions can be applied e.g. orally, topically, intravenously, intraperitoneally, subcutaneously and rectally in pharmaceutically effective

amounts.

In the following Examples, the anti-tumor active substances are tested by *in vitro* tests using intact tumor cells. Furthermore, a comparison of the activity test results and the tests evaluating the ability to penetrate cellular membranes shows that indigoid bisindole compounds which exhibit a good cell-penetrating ability show good to excellent anti-tumor activity.

The present invention is explained in detail by the following examples by which also further advantages of the present invention will become apparent.

EXAMPLES

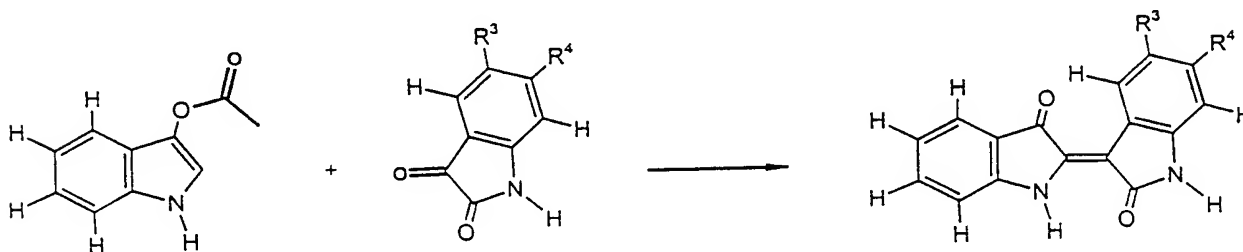
1. Synthesis of the compounds

The following general procedures were used to synthesize the indirubin derivatives according to Examples 1 to 69.

Synthesis of indirubines

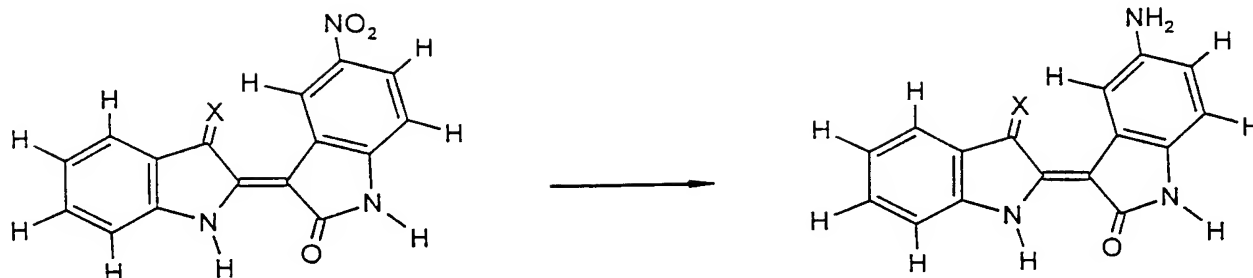
Method I

According to this method Examples 1-14 and 22-24 were prepared.



The indirubin derivatives are prepared according to Russel G. A., Kaupp G. (1969), *J. Am. Chem. Soc.*, **91**, pages 3851-3859.

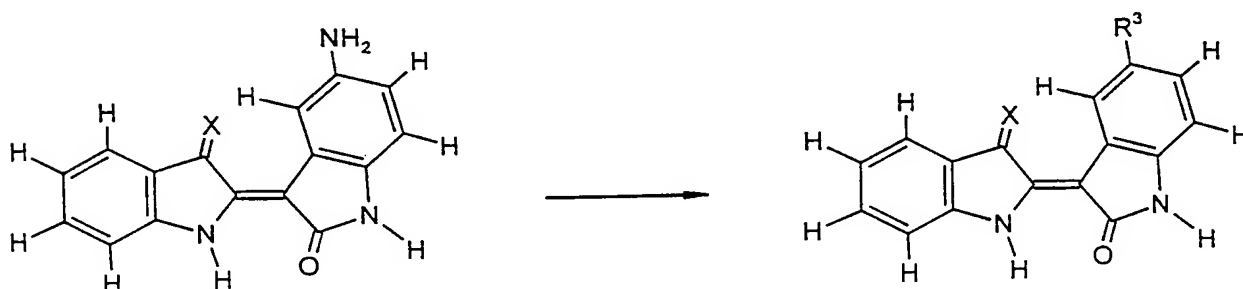
Method II



- 5 Example 15 is prepared by reducing 5-nitro-indirubin with iron / 0.2 N HCl in ethanol at 80 °C. The filtrate is neutralized by NaOH and stirred under aeration to reoxidize the leucobase to 5-amino-indirubin.

Method III

- 10 According to this method Examples 16-21 and 29-30 were prepared.

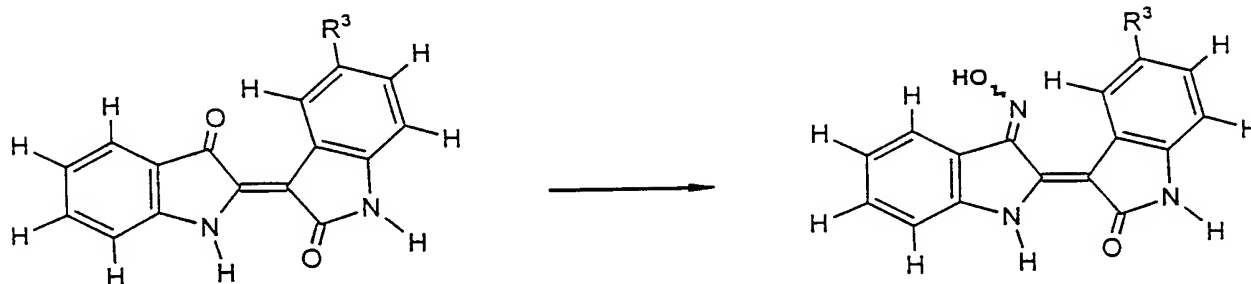


- 15 5-amino-indirubin or 5-amino-indirubin-3'-monooxime are suspended under stirring in pyridine containing catalytic amounts of a suitable base like N,N-dimethylaminopyridine, N-methylmorpholine or N,N,N,N-tetramethyl-guanidine. Using general methods of peptide chemistry, 5-amino-indirubin or 5-amino-indirubin-3'-monooxime is acylated with suitably activated acids, such as acyl chloride, acyl anhydrides or N-protected amino acid active esters, such as N-tert. butyloxycarbonyl (N-BOC)- or N-benzyloxycarbonyl (N-Z)-protected N-hydroxy-pyrrolidone-2,5-dione ester or the like. Deprotection is carried out with 0.2 N HCl /
- 20 formic acid or trifluoro acetic acid in the case of BOC derivatives and by catalytic hydrogenation in the case of Z-protected amino acids. In the latter case, indirubin leucobase is reoxidized by stirring under aeration.

Synthesis of indirubin-3'-monooximes

According to this method Examples 25-28 were prepared.

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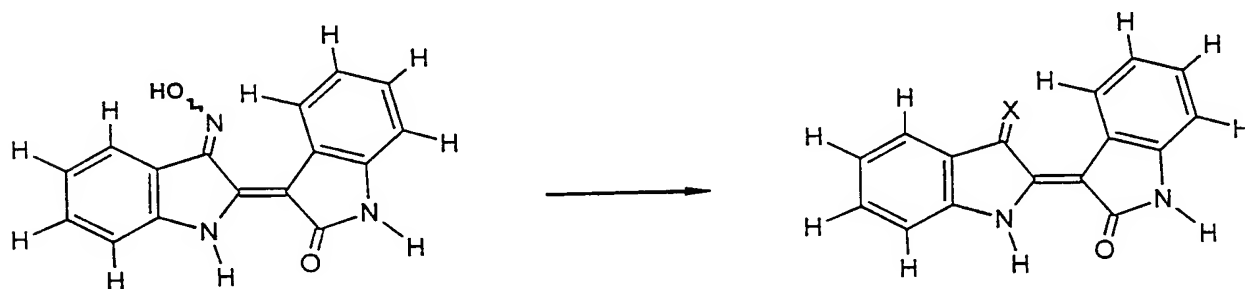


The indirubin-3'-monooxime derivatives were prepared as described in Farbwerke vorm. Meister Lucius & Brüning in Hoechst a. M. (1913), DRP 283726.

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Method I

According to this method Examples 31-49, 53, 54, 67 and 68 were prepared.



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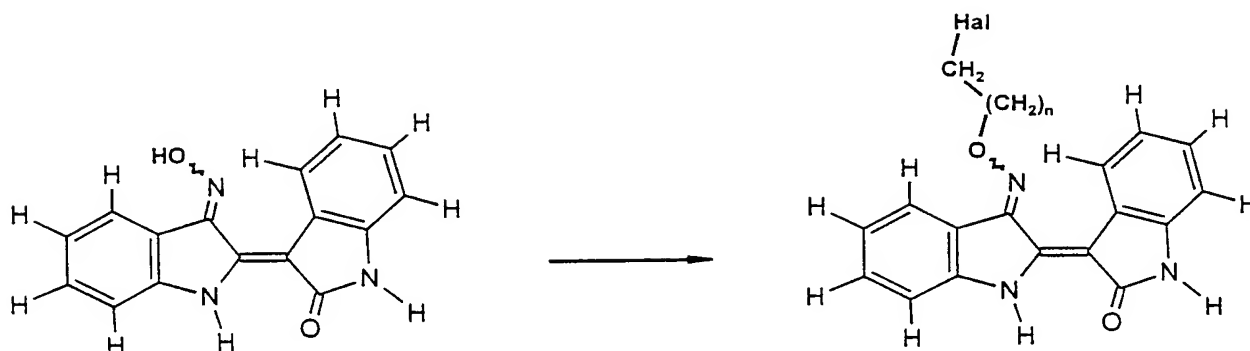
To a mixture of indirubin-3'-monooxime with 1-8 equivalents of a halogene alkane (Halogenes are Cl, Br or J) in a suitable solvent, such as tetrahydrofurane, dioxane, diglyme, methanol, ethanol, propanol or butanol, a suitable base is added (1-10 equivalents). The base is preferably N,N,N,N-tetramethyl-guanidine or other inorganic (KOH, NaOH) or organic bases (pyridine, triethylamine and the like). The reaction mixture is stirred for 0.5 to 10 h at room temperature or elevated temperature (up to 100 °C), depending on the solvent used. After termination of the reaction, the product precipitates and is filtered off.

20

25 Method II

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According to this method Examples 50-52 were prepared.



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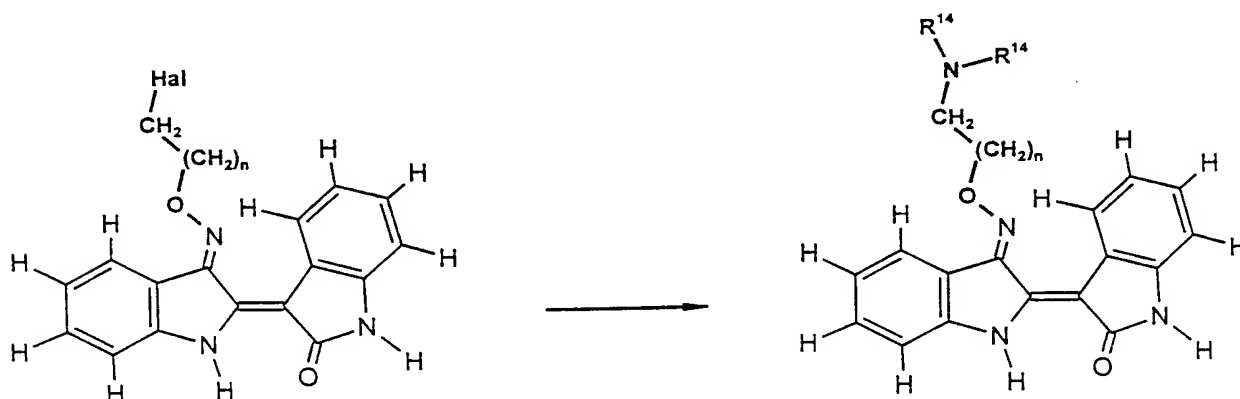
A solution of indirubin-3'-monooxime, N,N,N,N-tetramethyl-guanidine (1-4 equivalents) and ethanol is added dropwise to a solution of a suitable dihalogene alkane in ethanol. The dihalogene alkane preferably is 1,2- to 1,18-dibromo alkane or dichloro alkane. The precipitated product is filtered off and washed with ethanol.

10

Method III

According to this method Examples 55-66 and 69 were prepared.

15



The indirubin-3'-monooxime-halogene-alkyl-ether is dissolved in dimethylsulfoxide or ethanol or a mixture of both. The appropriate amine is added stepwise and the reaction mixture stirred for 5-24 h at room temperature or elevated temperature (up to 150 °C), depending on the solvent and the amino compound.

20

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Method IV

Example 53 and 54 were prepared as described by Method I. The N-acylated aminoglycoside was deprotected using general methods of carbohydrate chemistry. Removal of the N-acyl group was carried out using acetyl bromide as described by Micheel, Lengsfeld, Chem. Ber., 89, 1246-1250, 1956.

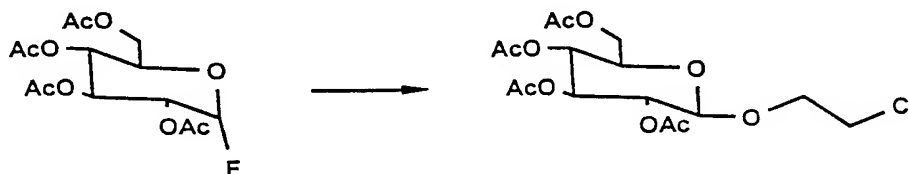
O-acetyl groups were removed with sodium ethanolate.

10 **Synthesis of the Halogenealkane-o-glycoside-ethers**

The halogenealkane-o-glycoside-ethers are prepared according to common methods of carbohydrate chemistry.

15 **Method I**

According to this method Examples A-F were prepared.



20 In accordance to R. Miethchen and V. Fehring, Liebigs Ann./Recueil, 553-561, 1997.

A solution of 2,3,4,6-tetra-O-acetyl- α -D-glucopyranosylfluoride (3.26 mmol) and 2-chloroethanol (3.35 mmol) in acetonitrile is mixed with 0.5 ml $F_3B \cdot Et_2O$ and stirring is continued for 30 min. After addition of dichloromethane (60 ml), the mixture is filtered trough silica gel. The filtrate is washed with aqueous $NaHCO_3$ and water. The dried organic phase is evaporated under reduced pressure and the compound recrystallized from hexane.

30

Method II

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According to this method Example G was prepared.



- 5 This synthesis follows a procedure described by L. Hough, K. C. McCarthy, A. C. Richardson, Recl. Trav. Chim. Pays-Bas, 110, 450 - 458, 1991. A mixture of L-arabinose (33 mmol), 2-chloroethanol (20 ml) and acetyl chloride (0.5 ml) was stirred at 60 °C for 24 h. The mixture was allowed to cool to room temperature and the crystalline precipitate filtered off and washed with acetone.

10

Identity and homogeneity of the compounds were established by elemental analysis and thin layer chromatography.

Where necessary, structural confirmation was obtained by NMR (¹H and ¹³C).

15

Example A (2-chloroethyl-2,3,4,6-tetraacetyl-D-glucopyranoside)

Yield: 79 %, fine needles

CHN-analysis: C₁₆H₂₃O₁₀Cl (MW: 410.81 g/mol)

20

calc.: 46.7 % C, 5.64% H,

found: 46.8 % C, 5.7 % H,

Example B (2-chloroethyl-2,3,4,6-tetraacetyl-D-galactopyranoside)

Yield: 76 %, fine needles

25 CHN-analysis: C₁₆H₂₃O₁₀Cl (MW: 410.81 g/mol)

calc.: 46.7 % C, 5.6 % H,

found: 46.8 % C, 5.7 % H,

Example C (3-chloropropyl-2,3,4,6-tetraacetyl-D-glucopyranoside)

30 Yield: 74 %, amorphous solid

CHN-analysis: C₁₇H₂₅O₁₀Cl (MW: 424.83 g/mol)

-15-

calc.: 48.0 % C, 5.9 % H,

found: 48.1 % C, 5.9 % H,

5 Example D (2-chloroethyl-2,3,6,8,9,10,12-heptaacetyl-O⁴-α-D-glucopyranosyl-D-glucopyranoside)

Yield: 76 %, amorphous solid

CHN-analysis: C₂₈H₃₉O₁₈Cl (MW: 699.06 g/mol)

calc.: 48.1 % C, 5.6 % H,

found: 48.2 % C, 5.7 % H,

10

Example E (2-chloroethyl-N,3,4,6-tetraacetyl-2-deoxy-amino-D-glucopyranoside)

Yield: 74 %, fine needles

CHN-analysis: C₁₆H₂₄O₉NCl (MW: 409.82 g/mol)

calc.: 46.9 % C, 5.9 % H, 3.42 % N

15

found: 46.9 % C, 5.9 % H, 3.51 % N

Example F (2-chloroethyl-N,3,4,6-tetraacetyl-2-deoxy-amino-D-galactopyranoside)

Yield: 72 %, fine needles

CHN-analysis: C₁₆H₂₄O₉NCl (MW: 409.82 g/mol)

20

calc.: 46.9 % C, 5.9 % H, 3.4 % N

found: 47.0 % C, 6.1 % H, 3.5 % N

Example G (2-chloroethyl-L-arabinose)

Yield: 24 %, fine needles

25 CHN-analysis: C₇H₁₃O₅Cl (MW: 212.63 g/mol)

calc.: 39.5 % C, 6.2 % H

found: 39.6 % C, 6.4 % H

Example 1 (6-iodo-indirubin)

30 Yield: 68 %, fine deep-purple powder

CHN-analysis: C₁₆H₉IN₂O₂ (MW: 388.16 g/mol)

calc.: 49.5 % C, 2.3 % H, 7.2 % N

found: 49.3 % C, 2.1 % H, 7.1 % N

Example 2 (5-ethyl-indirubin)

Yield: 92 %, fine deep-purple powder
CHN-analysis: $C_{18}H_{14}N_2O_2$ (MW: 290.32 g/mol)
calc.: 74.5 % C, 4.9 % H, 9.7 % N
5 found: 74.2 % C, 4.8 % H, 9.5 % N

Example 3 (5-isopropyl-indirubin)

Yield: 94 %, fine deep-purple powder
CHN-analysis: $C_{19}H_{19}N_2O_2$ (MW: 304.35 g/mol)
10 calc.: 75.0 % C, 5.3 % H, 9.2 % N
found: 74.8 % C, 5.2 % H, 9.1 % N

Example 4 (5-n-propyl-indirubin)

Yield: 93 %, fine deep-purple powder
15 CHN-analysis: $C_{19}H_{19}N_2O_2$ (MW: 304.35 g/mol)
calc.: 75.0 % C, 5.3 % H, 9.2 % N
found: 74.9 % C, 5.3 % H, 9.1 % N

Example 5 (5-(carboxymethyl)-indirubin)

20 Yield: 66 %, fine deep-purple crystals
CHN-analysis: $C_{19}H_{16}N_2O_4$ (MW: 320.30 g/mol)
calc.: 67.5 % C, 3.8 % H, 8.8 % N
found: 67.2 % C, 3.6 % H, 8.8 % N

Example 6 (5-[2-(piperazin-1-yl)-ethan-2-one-1-yl]-indirubin)

Yield: 55 %, fine deep-purple crystals
CHN-analysis: $C_{22}H_{21}N_4O_3$ (MW: 389.43 g/mol)
25 calc.: 67.9 % C, 5.4 % H, 14.4 % N
found: 68.1 % C, 5.7 % H, 14.6 % N

30

Example 7 (5-[2-(morpholin-4-yl)-ethan-2-one-1-yl]-indirubin)

Yield: 53 %, fine deep-purple crystals
CHN-analysis: $C_{22}H_{20}N_3O_4$ (MW: 390.42 g/mol)
calc.: 67.7 % C, 5.2 % H, 10.8 % N

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found: 67.8 % C, 5.3 % H, 11.0 % N

Example 8 (N-(2-amino-ethyl)-2-[3-(3'-oxo-(2'H3'H)indol-2'-ylidene)-(2H3H)indol-2-one-5-yl]-acetamide)

- 5 Yield: 63 %, fine deep-purple crystals
CHN-analysis: $C_{20}H_{18}N_4O_3$ (MW: 362.39 g/mol)
calc.: 66.3 % C, 5.0 % H, 15.5 % N
found: 66.5 % C, 5.1 % H, 15.6 % N

10 Example 9 (N-methyl-2-[3-(3'-oxo-(2'H3'H)indol-2'-ylidene)-(2H3H)indol-2-one-5-yl]-acetamide)

- Yield: 61 %, fine deep-purple crystals
CHN-analysis: $C_{19}H_{15}N_3O_3$ (MW: 333.35 g/mol)
calc.: 68.5 % C, 4.5 % H, 12.6 % N
15 found: 68.4 % C, 4.5 % H, 12.5 % N

Example 10 (N,N-dimethyl-2-[3-(3'-oxo-(2'H3'H)indol-2'-ylidene)-(2H3H)indol-2-one-5-yl]-acetamide)

- Yield: 59 %, fine deep-purple crystals
20 CHN-analysis: $C_{20}H_{17}N_3O_3$ (MW: 347.37 g/mol)
calc.: 69.2 % C, 4.5 % H, 12.1 % N
found: 69.1 % C, 4.5 % H, 12.2 % N

Example 11 (2-{2-[3-(3'-oxo-(2'H3'H)indol-2'-ylidene)-(2H3H)indol-2-one-5-yl]-acetyl-amino}-acetic acid)

- 25 Yield: 55 %, fine deep-purple crystals
CHN-analysis: $C_{20}H_{15}N_3O_5$ (MW: 377.35 g/mol)
calc.: 63.7 % C, 4.0 % H, 11.1 % N
found: 63.7 % C, 4.1 % H, 11.0 % N

30

Example 12 (methyl-2-{2-[3-(3'-oxo-(2'H3'H)indol-2'-ylidene)-(2H3H)indol-2-one-5-yl]-acetyl-amino}-acetate)

- Yield: 57 %, fine deep-purple crystals
CHN-analysis: $C_{21}H_{17}N_3O_5$ (MW: 391.38 g/mol)

calc.: 64.5 % C, 4.4 % H, 10.7 % N

found: 64.4 % C, 4.5 % H, 10.8 % N

5 Example 13 ([3-(3'-oxo-(2'H3'H)indol-2'-ylidene)-(2H3H)indol-2-one-5-yl]-methyl-phosphonic acid)

Yield: 61 %, fine deep-purple crystals

CHN-analysis: C₁₇H₁₃N₂O₅P (MW: 356.28 g/mol)

calc.: 57.3 % C, 3.7 % H, 7.9 % N

found: 57.2 % C, 3.6 % H, 7.9 % N

10 Example 14 (diethyl-([3-(3'-oxo-(2'H3'H)indol-2'-ylidene)-(2H3H)indol-2-one-5-yl]-methyl)-phosphonate)

Yield: 57 %, fine deep-purple crystals

CHN-analysis: C₂₁H₂₁N₂O₅P (MW: 412.38 g/mol)

15 calc.: 61.2 % C, 5.1 % H, 6.8 % N

found: 61.2 % C, 5.2 % H, 6.9 % N

Example 15 (5-amino-indirubin)

Yield: 72 %, fine deep-purple crystals

20 CHN-analysis: C₁₆H₁₁N₃O₂ (MW: 277.28 g/mol)

calc.: 69.3 % C, 4.0 % H, 15.2 % N

found: 69.2 % C, 3.9 % H, 15.4 % N

Example 16 (5-acetylamino-indirubin)

25 Yield: 64 %, fine deep-purple crystals

CHN-analysis: C₁₈H₁₃N₃O₃ (MW: 319.32 g/mol)

calc.: 67.7 % C, 4.1 % H, 13.2 % N

found: 67.6 % C, 4.2 % H, 13.3 % N

30 Example 17 ([3-(3'-oxo-(2'H3'H)indol-2'-ylidene)-(2H3H)indol-2-one-5-yl]-succinamic acid)

Yield: 48 %, fine deep-purple crystals

CHN-analysis: C₂₀H₁₅N₃O₅ (MW: 377.35 g/mol)

calc.: 63.7 % C, 4.0 % H, 11.1 % N

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found: 63.6 % C, 4.1 % H, 11.1 % N

Example 18 (2-amino-N-[3-(3'-oxo-(2'H3'H)indol-2'-ylidene)-(2H3H)indol-2-one-5-yl]-acetamide)

- 5 Yield: 60 %, fine deep-purple crystals
CHN-analysis: $C_{18}H_{14}N_4O_3$ (MW: 334.33 g/mol)
calc.: 64.7 % C, 4.2 % H, 16.8 % N
found: 64.6 % C, 4.2 % H, 16.7 % N

10 Example 19 (2-amino-N-[3-(3'-oxo-(2'H3'H)indol-2'-ylidene)-(2H3H)indol-2-one-5-yl]-propionamide)

- Yield: 64 %, fine deep-purple crystals
CHN-analysis: $C_{19}H_{16}N_4O_3$ (MW: 348.36 g/mol)
calc.: 65.5 % C, 4.6 % H, 16.1 % N
15 found: 65.6 % C, 4.4 % H, 16.0 % N

Example 20 (5-(2-amino-ethyl)-amino-indirubin)

- Yield: 52 %, fine deep-purple crystals
CHN-analysis: $C_{18}H_{16}N_4O_2$ (MW: 348.36 g/mol)
20 calc.: 67.5 % C, 5.0 % H, 17.5 % N
found: 67.6 % C, 5.1 % H, 17.3 % N

Example 21 (5-(2-hydroxy-ethyl)-amino-indirubin)

- Yield: 55 %, fine deep-purple crystals
25 CHN-analysis: $C_{18}H_{15}N_3O_3$ (MW: 321.33 g/mol)
calc.: 67.3 % C, 4.7 % H, 13.1 % N
found: 67.5 % C, 4.8 % H, 13.0 % N

Example 22 (indirubin-5-sulfonic acid-(piperazin-1-yl-amide))

- 30 Yield: 42 %, fine deep-purple crystals
CHN-analysis: $C_{20}H_{18}N_4O_4S$ (MW: 410.45 g/mol)
calc.: 58.5 % C, 4.4 % H, 13.7 % N
found: 58.6 % C, 4.6 % H, 13.8 % N

Example 23 (indirubin-5-sulfonic acid-(morpholin-4-yl-amide))

Yield: 42 %, fine deep-purple crystals

CHN-analysis: $C_{20}H_{17}N_3O_5S$ (MW: 411.43 g/mol)

calc.: 58.4 % C, 4.2 % H, 10.2 % N

5 found: 58.5 % C, 4.4 % H, 10.3 % N

Example 24 (methyl-2-([3-(3'-oxo-(2'H3'H)indol-2'-ylidene)-(2H3H)indol-2-one-5-yl]-sulfonylamino)-acetate)

Yield: 39 %, fine deep-purple crystals

10 CHN-analysis: $C_{19}H_{15}N_3O_6S$ (MW: 413.41 g/mol)

calc.: 55.2 % C, 3.7 % H, 10.2 % N

found: 55.4 % C, 3.7 % H, 10.3 % N

Example 25 (5-methyl-indirubin-3'-monooxime)

15 Yield: 56 %, red crystals

CHN-analysis: $C_{17}H_{13}N_3O_2$ (MW: 291.31 g/mol)

calc.: 70.1 % C, 4.5 % H, 14.4 % N

found: 69.9 % C, 4.5 % H, 14.3 % N

20 Example 26 (5-ethyl-indirubin-3'-monooxime)

Yield: 91 %, red crystals

CHN-analysis: $C_{18}H_{15}N_3O_2$ (MW: 305.34 g/mol)

calc.: 70.8 % C, 5.0 % H, 13.8 % N

found: 70.6 % C, 4.9 % H, 13.8 % N

25

Example 27 (5-isopropyl-indirubin-3'-monooxime)

Yield: 42 %, red crystals

CHN-analysis: $C_{19}H_{17}N_3O_2$ (MW: 319.36 g/mol)

calc.: 71.5 % C, 5.4 % H, 13.2 % N

30 found: 71.2 % C, 5.2 % H, 13.1 % N

Example 28 (5-amino-indirubin-3'-monooxime)

Yield: 58 %, red crystals

CHN-analysis: $C_{16}H_{12}N_4O_2$ (MW: 292.30 g/mol)

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calc.: 65.8 % C, 4.1 % H, 19.2 % N

found: 65.7 % C, 4.1 % H, 19.1 % N

Example 29 (5-acetylamino-indirubin-3'-monooxime)

5 Yield: 52 %, red crystals

CHN-analysis: $C_{18}H_{14}N_4O_3$ (MW: 334.33 g/mol)

calc.: 64.7 % C, 4.2 % H, 16.8 % N

found: 64.7 % C, 4.1 % H, 16.9 % N

10 Example 30 (2-amino-N-[3-(3'-hydroxyimino-(2'H3'H)indol-2'-ylidene)-(2H3H)-indol-2-one-5-yl]-acetamide)

Yield: 62 %, red crystals

CHN-analysis: $C_{18}H_{15}N_5O_3$ (MW: 349.35 g/mol)

calc.: 61.9 % C, 4.3 % H, 20.1 % N

15 found: 62.0 % C, 4.1 % H, 19.9 % N

Example 31 (3-[3'-(iminooxy-O-(2-hydroxy-ethyl)-(2'H3'H)indol-2'-ylidene]-(2H3H)indol-2-one)

Yield: 79 %, red powder

20 CHN-analysis: $C_{18}H_{15}N_3O_3$ (MW: 321.33 g/mol)

calc.: 67.3 % C, 4.7 % H, 13.1 % N

found: 67.2 % C, 4.8 % H, 13.0 % N

25 Example 32 (3-[3'-(iminooxy-O-(3-hydroxy-propyl)-(2'H3'H)indol-2'-ylidene]-(2H3H)indol-2-one)

Yield: 83 %, red powder

CHN-analysis: $C_{19}H_{17}N_3O_3$ (MW: 335.36 g/mol)

calc.: 68.1 % C, 5.1 % H, 12.5 % N

30 found: 67.9 % C, 5.2 % H, 12.4 % N

Example 33 (3-[3'-(iminooxy-O-(2-(2-hydroxy-ethoxy)-ethyl)]-(2'H3'H)indol-2'-ylidene]-(2H3H)indol-2-one)

Yield: 64 %, red powder

CHN-analysis: $C_{20}H_{19}N_3O_4$ (MW: 365.39 g/mol)
calc.: 65.8 % C, 5.2 % H, 11.5 % N
found: 65.6 % C, 5.2 % H, 11.4 % N

5 Example 34 (3-{3'-[iminooxy-O-((2-hydroxy-2-methyl)-propyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)

Yield: 90 %, red powder

CHN-analysis: $C_{20}H_{19}N_3O_3$ (MW: 349.39 g/mol)
calc.: 68.8 % C, 5.5 % H, 12.0 % N
found: 68.7 % C, 5.5 % H, 12.0 % N

10 Example 35 (2-{O-[2'-(2-oxo-(2H3H)indol-3-ylidene)-(2'H3'H)indol-3'-ylidene]-aminooxy}-acetic acid (sodium salt))

Yield: 57 %, red powder

15 CHN-analysis: $C_{18}H_{12}N_3O_4Na$ (MW: 357.30 g/mol)
calc.: 60.5 % C, 3.4 % H, 11.8 % N
found: 60.3 % C, 3.6 % H, 11.7 % N

20 Example 36 (3-{O-[2'-(2-oxo-(2H3H)indol-3-ylidene)-(2'H3'H)indol-3'-ylidene]-aminooxy}-propionic acid (sodium salt))

Yield: 59 %, red powder

CHN-analysis: $C_{19}H_{14}N_3O_4Na$ (MW: 371.33 g/mol)
calc.: 61.5 % C, 3.8 % H, 11.3 % N
found: 61.4 % C, 3.6 % H, 11.4 % N

25 Example 37 (4-{O-[2'-(2-oxo-(2H3H)indol-3-ylidene)-(2'H3'H)indol-3'-ylidene]-aminooxy}-butyric acid (sodium salt))

Yield: 58 %, red powder

30 CHN-analysis: $C_{20}H_{16}N_3O_4Na$ (MW: 385.35 g/mol)
calc.: 62.3 % C, 4.2 % H, 10.9 % N
found: 62.0 % C, 4.3 % H, 11.0 % N

Example 38 (5-{O-[2'-(2-oxo-(2H3H)indol-3-ylidene)-(2'H3'H)indol-3'-ylidene]-aminooxy}-pentanoic acid (sodium salt))

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Yield: 52 %, red powder
CHN-analysis: $C_{21}H_{18}N_3O_4Na$ (MW: 399.38 g/mol)
calc.: 63.2 % C, 4.5 % H, 10.5 % N
found: 63.0 % C, 4.4 % H, 10.7 % N

5

Example 39 (3-[3'-(iminooxy-O-carbethoxy)-(2'H3'H)indol-2'-ylidene]-(2H3H)indol-2-one)

Yield: 82 %, red powder
CHN-analysis: $C_{19}H_{15}N_3O_4$ (MW: 349.34 g/mol)
calc.: 65.3 % C, 4.3 % H, 12.0 % N
found: 65.3 % C, 4.4 % H, 11.9 % N

10

Example 40 (ethyl-2-{O-[2'-(2-oxo-(2H3H)indol-3-ylidene)-(2'H3'H)indol-3'-ylidene]-aminooxy}-acetate)

Yield: 31 %, red powder
CHN-analysis: $C_{20}H_{17}N_3O_4$ (MW: 363.37 g/mol)
calc.: 66.1 % C, 4.7 % H, 11.6 % N
found: 66.2 % C, 4.6 % H, 11.5 % N

15

Example 41 (3-{3'-[iminooxy-O-((N,N)-dimethyl-carbamoyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)

Yield: 89 %, red powder
CHN-analysis: $C_{19}H_{16}N_4O_3$ (MW: 348.36 g/mol)
calc.: 65.5 % C, 4.6 % H, 16.1 % N
found: 65.2 % C, 4.5 % H, 15.9 % N

25

Example 42 (2-{O-[2'-(2-oxo-(2H3H)indol-3-ylidene)-(2'H3'H)indol-3'-ylidene]-aminooxy}-acetamide)

Yield: 86 %, red powder
CHN-analysis: $C_{18}H_{14}N_4O_3$ (MW: 334.33 g/mol)
calc.: 64.7 % C, 4.2 % H, 16.8 % N
found: 64.8 % C, 4.4 % H, 16.5 % N

30

Example 43 (N,N-dimethyl-2-{O-[2'-(2-oxo-(2H3H)indol-3-ylidene)-(2'H3'H)indol-

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3'-ylidene]-aminooxy)-acetamide)

Yield: 89 %, red powder

CHN-analysis: $C_{18}H_{14}N_4O_3$ (MW: 362.39 g/mol)

calc.: 66.3 % C, 5.0 % H, 15.5 % N

5 found: 66.1 % C, 4.7 % H, 15.5 % N

Example 44 (2-{2-[O-(2'-(2-oxo-(2H3H)indol-3-ylidene)-(2'H3'H)indol-3'-ylidene)-aminooxy]-acetyl-amino}-acetic acid)

Yield: 79 %, red powder

10 CHN-analysis: $C_{20}H_{16}N_4O_5$ (MW: 392.37 g/mol)

calc.: 61.2 % C, 4.1 % H, 14.3 % N

found: 61.3 % C, 4.0 % H, 14.1 % N

Example 45 (3-{3'-[iminooxy-O-(3-D-glucopyranosylpropyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)

15

Yield: 28 %, red powder

CHN-analysis: $C_{25}H_{25}N_3O_8$ (MW: 495.49 g/mol)

calc.: 60.6 % C, 5.1 % H, 8.5 % N

found: 60.8 % C, 5.2 % H, 8.6 % N

20

Example 46 (3-{3'-[iminooxy-O-(O⁴- α -D-glucopyranosyl-2-D-glucopyranosylethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)

Yield: 20 %, red powder

CHN-analysis: $C_{31}H_{34}N_3O_{13}$ (MW: 656.62 g/mol)

25 calc.: 56.7 % C, 5.2 % H, 6.4 % N

found: 56.9 % C, 5.3 % H, 6.5 % N

Example 47 (3-{3'-[iminooxy-O-(2-D-galactopyranosylethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)

30 Yield: 32 %, red powder

CHN-analysis: $C_{24}H_{25}N_3O_8$ (MW: 483.48g/mol)

calc.: 56.5 % C, 5.5 % H, 8.2 % N

found: 56.7 % C, 5.3 % H, 8.3 % N

Example 48 (3-{3'-[iminooxy-O-(2-D-glucopyranosylethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)

Yield: 41 %, red powder

CHN-analysis: C₂₄H₂₅N₃O₈ (MW: 483.48 g/mol)

5 calc.: 56.5 % C. 5.5 % H. 8.2 % N

found: 56.7 % C. 5.4 % H. 8.3 % N

Example 49 (3-{3'-[iminooxy-O-(2-L-arabinopyranosylethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)

10 Yield: 43 %, red powder

CHN-analysis: C₂₃H₂₂N₃O₇ (MW: 452.44 g/mol)

calc.: 61.1 % C. 4.9 % H. 9.3 % N

found: 61.2 % C. 5.0 % H. 9.4 % N

15 Example 50 (3-{3'-[iminooxy-O-(2-chloroethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)

Yield: 97 %, red needles

CHN-analysis: C₁₈H₁₄N₃O₂Cl (MW: 339.78 g/mol)

calc.: 63.6 % C. 4.2 % H. 12.4 % N

20 found: 63.8 % C. 4.4 % H. 12.5 % N

Example 51 (3-{3'-[iminooxy-O-(4-chlorobutyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)

Yield: 96 %, red needles

25 CHN-analysis: C₂₀H₁₈N₃O₂Cl (MW: 367.83 g/mol)

calc.: 65.3 % C. 4.9 % H. 11.4 % N

found: 65.5 % C. 4.8 % H. 12.5 % N

Example 52 (3-{3'-[iminooxy-O-(10-chlorodecyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)

30

Yield: 95 %, red needles

CHN-analysis: C₂₆H₃₀N₃O₂Cl (MW: 452.00 g/mol)

calc.: 69.1 % C. 6.7 % H. 9.3 % N

found: 69.3 % C. 6.8 % H. 9.4 % N

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Example 53 (3-{3'-[iminooxy-O-(2-(2-amino-2-deoxy-D-glucopyranosyl)-ethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)

Yield: 29 %, red powder

5 CHN-analysis: $C_{24}H_{26}N_4O_7$ (MW: 482.49 g/mol)
calc.: 59.7 % C. 5.4 % H. 11.6 % N
found: 59.9 % C. 5.4 % H. 11,7 % N

Example 54 (3-{3'-[iminooxy-O-(2-(2-amino-2-deoxy-D-galactopyranosyl)-ethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)

Yield: 26 %; red powder

CHN-analysis: $C_{24}H_{26}N_4O_7$ (MW: 482.49 g/mol)
calc.: 59.7 % C. 5.4 % H. 11.6 % N
found: 59.9 % C. 5.6 % H. 11,7 % N

15

Example 55 (3-{3'-[iminooxy-O-(N-(1-deoxy-glucitol)-2-amino-ethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)

Yield: 43 %, red powder

20 CHN-analysis: $C_{24}H_{28}N_4O_7$ (MW: 484.51 g/mol)
calc.: 59.5 % C. 5.8 % H. 11.6 % N
found: 59.6 % C. 6.0 % H. 11.7 % N

Example 56 (3-{3'-[iminooxy-O-(N-(2-deoxy-glucose)-2-amino-ethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)

25 Yield: 48 %, red powder

CHN-analysis: $C_{24}H_{26}N_4O_7$ (MW: 482.49 g/mol)
calc.: 59.7 % C. 5.4 % H. 11.6 % N
found: 59.9 % C. 5.5 % H. 11,8 % N

Example 57 (3-{3'-[iminooxy-O-(N-(2-deoxy-galactose)-2-amino-ethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)

Yield: 46 %, red powder

30 CHN-analysis: $C_{24}H_{26}N_4O_7$ (MW: 482.49 g/mol)
calc.: 59.7 % C. 5.4 % H. 11.6 % N

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found: 59.8 % C. 5.4 % H. 11,7 % N

Example 58 (3-{3'-[iminooxy-O-((N,N)-dimethyl-2-amino-ethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)

- 5 Yield: 48 %, red powder
CHN-analysis: C₂₀H₂₀N₄O₂ (MW: 348.40 g/mol)
calc.: 69.0 % C. 6.8 % H. 16.1 % N
found: 69.2 % C. 6.9 % H. 16.2 % N

10 Example 59 (3-{3'-[iminooxy-O-(N-hydroxyethyl-2-amino-ethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)

- Yield: 47 %, red powder
CHN-analysis: C₂₀H₂₀N₄O₃ (MW: 364.40 g/mol)
calc.: 65.9 % C. 5.5 % H. 15.4 % N
found: 66.1 % C. 5.6 % H. 15.5 % N

15

Example 60 (3-{3'-[iminooxy-O-((N,N)-bis(hydroxyethyl)-2-amino-ethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)

- Yield: 47 %, red powder
20 CHN-analysis: C₂₂H₂₄N₄O₄ (MW: 408.46 g/mol)
calc.: 64.7 % C. 5.9 % H. 13.7 % N
found: 64.9 % C. 6.1 % H. 13.8 % N

Example 61 (3-{3'-[iminooxy-O-((N,N)-bis(hydroxyethyl)-4-amino-butyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)

- 25 Yield: 46 %, red powder
CHN-analysis: C₂₆H₃₂N₄O₇ (MW: 512.56 g/mol)
calc.: 60.9 % C. 6.3 % H. 10.9 % N
found: 61.1 % C. 6.4 % H. 11.0 % N

30

Example 62 (3-{3'-[iminooxy-O-((N,N)-bis(hydroxyethyl)-10-amino-decyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)

- Yield: 48 %, red powder
CHN-analysis: C₃₂H₄₄N₄O₇ (MW: 596.72 g/mol)

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calc.: 64.4 % C. 7.4 % H. 9.4 % N

found: 64.5 % C. 7.5 % H. 9.4 % N

5 Example 63 (3-{3'-[iminooxy-O-(2-(piperazin-1-yl)-ethyl)]-(2'H3'H)indol-2'-ylidene}-
(2H3H)indol-2-one)

Yield: 47 %, red powder

CHN-analysis: C₂₂H₂₃N₅O₂ (MW: 389.46 g/mol)

calc.: 67.8 % C. 5.9 % H. 18.0 % N

found: 67.9 % C. 6.1 % H. 18.1 % N

10 Example 64 (3-{3'-[iminooxy-O-(2-(morpholin-4-yl)-ethyl)]-(2'H3'H)indol-2'-ylidene}-
(2H3H)indol-2-one)

Yield: 48 %, red powder

CHN-analysis: C₂₂H₂₃N₄O₃ (MW: 391.45 g/mol)

15 calc.: 67.5 % C. 5.9 % H. 14.3 % N

found: 67.6 % C. 6.1 % H. 14.5 % N

Example 65 (3-{3'-[iminooxy-O-(2-(4-methyl-piperazin-1-yl)-ethyl)]-(2'H3'H)indol-2'-
ylidene}-(2H3H)indol-2-one)

20 Yield: 47 %, red powder

CHN-analysis: C₂₃H₂₅N₅O₂ (MW: 403.48 g/mol)

calc.: 68.5% C. 6.3 % H. 17.4 % N

found: 68.7% C. 6.2 % H. 17.3 % N

25 Example 66 (3-{3'-[iminooxy-O-(2-(2-amino-ethyl)-amino-ethyl)]-(2'H3'H)indol-2'-
ylidene}-(2H3H)indol-2-one)

Yield: 46 %, red needles

CHN-analysis: C₂₀H₂₁N₂O₂ (MW: 321.40g/mol)

calc.: 74.7 % C. 6.6 % H. 8.7 % N

30 found: 74.9 % C. 6.8 % H. 8.8 % N

Example 67 (3-[3'-[iminooxy-O-(2-hydroxy-ethyl)]-(2H'3H')indol-2'-ylidene]-5-
methyl-(2H3H)indol-2-one)

Yield: 77 %, red powder

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CHN-analysis: $C_{19}H_{17}N_3O_3$ (MW: 335.36 g/mol)
 calc.: 68.1 % C, 5.1 % H, 12.5 % N
 found: 68.2 % C, 5.2 % H, 12.6 % N

5 Example 68 (3-[3'-[iminooxy-O-(2-D-glucopyranosylethyl)]-(2H'3H')indol-2'-ylidene]-5-methyl-(2H3H)indol-2-one)

Yield: 44 %, red powder

CHN-analysis: $C_{25}H_{27}N_3O_8$ (MW: 497.50 g/mol)
 calc.: 60.4 % C, 5.5 % H, 8.5 % N
 10 found: 60.6 % C, 5.6 % H, 8.7 % N

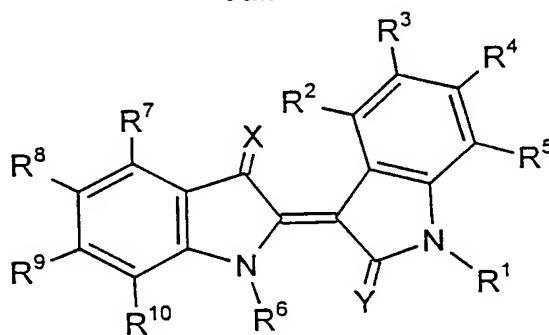
Example 69 (3-[3'-[iminooxy-O-(N-(1-deoxy-glucitol)-2-amino-ethyl)]-(2H'3H')indol-2'-ylidene]-5-methyl-(2H3H)indol-2-one)

Yield: 43 %, red powder

15 CHN-analysis: $C_{25}H_{30}N_4O_7$ (MW: 498.54 g/mol)
 calc.: 60.2 % C, 6.1 % H, 11.2 % N
 found: 60.4 % C, 6.2 % H, 11.4 % N

Table 1 summarizes the structures of the indirubin compounds of Examples 1 to
 20 30.

Table 1



Example	R ³	R ⁴	X
1	H	I	O
2	CH ₂ -CH ₃	H	O
3	CH(CH ₃) ₂	H	O
4	CH ₂ -CH ₂ -CH ₃	H	O

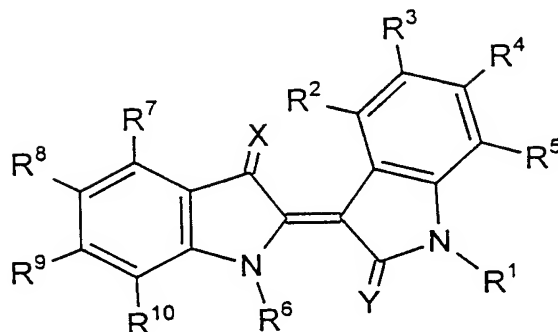
Table 1 (continued)

Example	R ³	R ⁴	X
5	CH ₂ -COOH	H	O
6	CH ₂ -CO-(NC ₄ H ₈ NH)	H	O
7	CH ₂ -CO-(NC ₄ H ₈ O)	H	O
8	CH ₂ -CO-NH(CH ₂ -CH ₂ -NH ₂)	H	O
9	CH ₂ -CO-NH(CH ₃)	H	O
10	CH ₂ -CO-N(CH ₃) ₂	H	O
11	CH ₂ -CO-NH(CH ₂ -COOH)	H	O
12	CH ₂ -CO-NH(CH ₂ -CO-O-CH ₃)	H	O
13	CH ₂ -PO(OH) ₂	H	O
14	CH ₂ -PO(O-CH ₂ -CH ₃) ₂	H	O
15	NH ₂	H	O
16	NH-CO-CH ₃	H	O
17	NH-CO-CH ₂ -CH ₂ -COOH	H	O
18	NH-CO-CH ₂ -NH ₂	H	O
19	NH-CO-CH(CH ₃)-NH ₂	H	O
20	NH-CH ₂ -CH ₂ -NH ₂	H	O
21	NH-CH ₂ -CH ₂ -OH	H	O
22	SO ₂ -(NC ₄ H ₈ NH)	H	O
23	SO ₂ -(NC ₄ H ₈ O)	H	O
24	SO ₂ -NH(CH ₂ -CO-O-CH ₃)	H	O
25	CH ₃	H	NOH
26	CH ₂ -CH ₃	H	NOH
27	CH(CH ₃) ₂	H	NOH
28	NH ₂	H	NOH
29	NH-CO-CH ₃	H	NOH
30	NH-CO-CH ₂ -NH ₂	H	NOH

In Table 1, (NC₄H₈NH) represents a piperazino group and (NC₄H₈O) represents a morpholino group, and R¹, R², R⁵ and R⁶ - R¹⁰ represent a hydrogen atom and Y represents an oxygen atom.

Table 2 summarizes the structures of the indirubin compounds of Examples 31 to 69.

Table 2



5

Example	R ³	R ⁴	X
31	H	H	NO-CH ₂ -CH ₂ -OH
32	H	H	NO-CH ₂ -CH ₂ -CH ₂ -OH
33	H	H	NO-CH ₂ -CH ₂ -O-CH ₂ -CH ₂ -OH
34	H	H	NO-CH ₂ -C(OH)(CH ₃) ₂
35	H	H	NO-CH ₂ -COO ⁻ Na ⁺
36	H	H	NO-CH ₂ -CH ₂ -COO ⁻ Na ⁺
37	H	H	NO-CH ₂ -CH ₂ -CH ₂ -COO ⁻ Na ⁺
38	H	H	NO-CH ₂ -CH ₂ -CH ₂ -CH ₂ -COO ⁻ Na ⁺
39	H	H	NO-CO-O-CH ₂ -CH ₃
40	H	H	NO-CH ₂ -CO-O-CH ₂ -CH ₃
41	H	H	NO-CO-N(CH ₃) ₂
42	H	H	NO-CH ₂ -CO-NH ₂
43	H	H	NO-CH ₂ -CO-N(CH ₃) ₂
44	H	H	NO-CH ₂ -CO-NH-CH ₂ -COOH
45	H	H	NO-CH ₂ -CH ₂ -CH ₂ -O-glucose
46	H	H	NO-CH ₂ -CH ₂ -O-maltose
47	H	H	NO-CH ₂ -CH ₂ -O-galactose
48	H	H	NO-CH ₂ -CH ₂ -O-glucose
49	H	H	NO-CH ₂ -CH ₂ -O-arabinose
50	H	H	NO-CH ₂ -CH ₂ -Cl
51	H	H	NO-(CH ₂) ₄ -Cl
52	H	H	NO-(CH ₂) ₁₀ -Cl
53	H	H	NO-CH ₂ -CH ₂ -O-glucosamine

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Table 2 (continued)

Example	R ³	R ⁴	X
54	H	H	NO-CH ₂ -CH ₂ -O-galactosamine
55	H	H	NO-CH ₂ -CH ₂ -NH-CH ₂ -(CHOH) ₄ -CH ₂ -OH
56	H	H	NO-CH ₂ -CH ₂ -NH-(2-deoxy-glucose)
57	H	H	NO-CH ₂ -CH ₂ -NH-(2-deoxy-galactose)
58	H	H	NO-CH ₂ -CH ₂ -N(CH ₃) ₂
59	H	H	NO-CH ₂ -CH ₂ -NH-CH ₂ -CH ₂ -OH
60	H	H	NO-CH ₂ -CH ₂ -N(CH ₂ -CH ₂ -OH) ₂
61	H	H	NO-(CH ₂) ₄ -N(CH ₂ -CH ₂ -OH) ₂
62	H	H	NO-(CH ₂) ₁₀ -N(CH ₂ -CH ₂ -OH) ₂
63	H	H	NO-CH ₂ -CH ₂ -N(CH ₂ -CH ₂) ₂ NH
64	H	H	NO-CH ₂ -CH ₂ -N(CH ₂ -CH ₂) ₂ O
65	H	H	NO-CH ₂ -CH ₂ -N(CH ₂ -CH ₂) ₂ N-CH ₃
66	H	H	NO-CH ₂ -CH ₂ -NH-CH ₂ -CH ₂ -NH
67	CH ₃	H	NO-CH ₂ -CH ₂ -OH
68	CH ₃	H	NO-CH ₂ -CH ₂ -O-glucose
69	CH ₃	H	NO-CH ₂ -CH ₂ -NH-CH ₂ -(CHOH) ₄ -CH ₂ -OH

- 5 In Table 2, N(CH₂CH₂)₂NH represents a piperazino group and N(CH₂CH₂)₂O represents a morpholino group, R¹ - R⁵ and R⁶ - R¹⁰ represent a hydrogen atom and Y represents an oxygen atom.

2. Cellular uptake

Compounds 2, 15, 25, 26, 28, 31, 48 and 55 were examined with respect to their uptake by LXFL-529L cells. Results are shown in Table 3. The amounts of the substances which were taken up by the cells are given depending on the concentration of the substance within the incubation medium. The time of incubation was 2 hours in all of the experiments. Furthermore, distribution between cytosol and the cellular organelles (particular) was measured and is given in the last two columns of Table 3.

Table 3

Compound	concentration of incubation [μ M]	amount taken up [μ g/mg protein]	distribution	
			cytosol [%]	particular [%]
2	10	0.20 ± 0.05	10 ± 2	90 ± 6
	20	0.22 ± 0.07	5 ± 2	95 ± 3
15	10	0.25 ± 0.04	15 ± 2	85 ± 4
	20	0.29 ± 0.05	6 ± 1	94 ± 4
25	10	0.12 ± 0.03	20 ± 4	80 ± 5
	20	0.15 ± 0.04	16 ± 3	84 ± 4
	50	0.18 ± 0.06	10 ± 4	90 ± 8
26	10	0.10 ± 0.03	18 ± 3	82 ± 6
	20	0.13 ± 0.05	11 ± 3	89 ± 5
28	10	0.20 ± 0.06	10 ± 2	90 ± 6
	20	0.23 ± 0.05	90 ± 6	94 ± 5
31	10	0.27 ± 0.06	7 ± 1	93 ± 7
	20	0.31 ± 0.07	7 ± 2	93 ± 6

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Table 3 (continued)

Compound	concentration of incubation [μM]	amount taken up [$\mu\text{g}/\text{mg}$ protein]	distribution	
			cytosol [%]	particular [%]
48	10	0.20 ± 0.05	13 ± 3	87 ± 7
	20	0.25 ± 0.04	5 ± 1	95 ± 6
55	10	0.37 ± 0.07	9 ± 2	91 ± 5
	20	0.42 ± 0.06	7 ± 1	93 ± 6

5

3. Evaluation of tumor cell growth inhibition

Examples 1-49 and 53-69 were tested with respect to their anti-tumor activity via a standard cellular growth inhibition assay (SRB-assay) using cells of the large-cell lung carcinoma of the tumor line LXFL-529L and cells of the mammary carcinoma cell line MCF-7. The results are shown in Table 4. Tumor cell growth inhibition was determined by the sulfo-rhodamine B-assay (SRB-assay) according to Skehan et al. (1990), *J. Natl. Cancer Institute* **82**, pages 1107-1112. Incubation was for three days in serum-containing medium. Results are given as IC_{50} -values defined as the concentration of compound [μM] inducing 50 % growth inhibition, compared to vehicle treated control.

Table 4

Example	SRB-Assay IC_{50} [μM]	
	LXFL-529L	MCF-7
1 (6-iodo-indirubin)	15.0 ± 0.5	15.0 ± 0.8
2 (5-ethyl-indirubin)	7.0 ± 0.2	7.0 ± 0.2
3 (5-isopropyl-indirubin)	4.0 ± 0.2	0.5 ± 0.2
4 (5-n-propyl-indirubin)	7.0 ± 0.4	6.5 ± 0.5
5 (5-(carboxymethyl)-indirubin)	18.0 ± 1.0	19.5 ± 1.4

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Table 4 (continued)

Example	SRB-Assay IC ₅₀ [μM]	
	LXFL-529L	MCF-7
6 (5-[2-(piperazin-1-yl)-ethan-2-one-1-yl]-indirubin)	4.0 ± 0.5	8.0 ± 0.9
7 (5-[2-(morpholin-4-yl)-ethan-2-one-1-yl]-indirubin)	5.5 ± 0.7	7.5 ± 0.6
8 (N-(2-amino-ethyl)-2-[3-(3'-oxo-(2'H3'H)indol-2'-ylidene)-(2H3H)indol-2-one-5-yl]-acetamide)	3.5 ± 0.7	4.0 ± 0.6
9 (N-methyl-2-[3-(3'-oxo-(2'H3'H)indol-2'-ylidene)-(2H3H)indol-2-one-5-yl]-acetamide)	11.5 ± 0.6	17.0 ± 1.0
10 (N,N-dimethyl-2-[3-(3'-oxo-(2'H3'H)indol-2'-ylidene)-(2H3H)indol-2-one-5-yl]-acetamide)	16.0 ± 0.9	20.0 ± 1.3
11 (2-[2-[3-(3'-oxo-(2'H3'H)indol-2'-ylidene)-(2H3H)indol-2-one-5-yl]-acetylamino]-acetic acid)	15.0 ± 0.8	20.5 ± 0.6
12 (methyl-2-[2-[3-(3'-oxo-(2'H3'H)indol-2'-ylidene)-(2H3H)indol-2-one-5-yl]-acetylamino]-acetate)	8.0 ± 0.8	6.5 ± 1.2
13 ([3-(3'-oxo-(2'H3'H)indol-2'-ylidene)-(2H3H)indol-2-one-5-yl]-methyl-phosphonic acid)	8.5 ± 0.8	6.5 ± 1.0
14 (diethyl-{[3-(3'-oxo-(2'H3'H)indol-2'-ylidene)-(2H3H)indol-2-one-5-yl]-methyl}-phosphonate)	6.5 ± 1.2	6.0 ± 1.1
15 (5-amino-indirubin)	8.0 ± 0.7	5.0 ± 0.5
16 (5-acetylamino-indirubin)	10.0 ± 0.8	15.0 ± 1.0
17 ([3-(3'-oxo-(2'H3'H)indol-2'-ylidene)-(2H3H)indol-2-one-5-yl]-succinamic acid)	12.5 ± 0.7	14.0 ± 0.9
18 (2-amino-N-[3-(3'-oxo-(2'H3'H)indol-2'-ylidene)-(2H3H)indol-2-one-5-yl]-acetamide)	4.0 ± 0.4	3.0 ± 0.3

Table 4 (continued)

Example	SRB-Assay IC ₅₀ [μM]	
	LXFL-529L	MCF-7
19 (2-amino-N-[3-(3'-oxo-(2'H3'H)indol-2'-ylidene)-(2H3H)indol-2-one-5-yl]-propionamide)	7.0 ± 0.7	5.0 ± 0.6
20 (5-(2-amino-ethyl)-amino-indirubin)	4.0 ± 0.3	3.5 ± 0.4
21 (5-(2-hydroxy-ethyl)-amino-indirubin)	3.0 ± 0.5	5.0 ± 0.6
22 (indirubin-5-sulfonic acid-(piperazin-1-yl-amide))	3.0 ± 0.5	2.0 ± 0.3
23 (indirubin-5-sulfonic acid-(morpholin-4-yl-amide))	4.5 ± 0.8	3.0 ± 0.9
24 (methyl-2-[[3-(3'-oxo-(2'H3'H)indol-2'-ylidene)-(2H3H)indol-2-one-5-yl]-sulfonylamino]-acetate)	16.0 ± 0.8	25.0 ± 0.9
25 (5-methyl-indirubin-3'-monooxime)	6.0 ± 0.8	6.0 ± 0.9
26 (5-ethyl-indirubin-3'-monooxime)	6.0 ± 0.6	7.0 ± 0.9
27 (5-isopropyl-indirubin-3'-monooxime)	5.5 ± 0.6	5.0 ± 0.9
28 (5-amino-indirubin-3'-monooxime)	7.5 ± 0.4	5.0 ± 0.8
29 (5-acetylamino-indirubin-3'-monooxime)	4.0 ± 0.9	5.0 ± 0.5
30 (2-amino-N-[3-(3'-hydroxyimino-(2'H3'H)indol-2'-ylidene)-(2H3H)indol-2-one-5-yl]-acetamide)	6.0 ± 0.8	6.0 ± 0.7
31 (3-[3'-(iminooxy-O-(2-hydroxy-ethyl)-(2'H3'H)indol-2'-ylidene)]-(2H3H)indol-2-one)	1.5 ± 0.4	2.5 ± 0.4
32 (3-[3'-(iminooxy-O-(3-hydroxy-propyl)-(2'H3'H)indol-2'-ylidene)]-(2H3H)indol-2-one)	1.5 ± 0.3	2.0 ± 0.4
33 (3-[3'-[iminooxy-O-(2-(2-hydroxy-ethoxy)-ethyl)]-(2'H3'H)indol-2'-ylidene]]-(2H3H)indol-2-one)	2.0 ± 0.4	2.5 ± 0.5

Table 4 (continued)

Example	SRB-Assay IC ₅₀ [μM]	
	LXFL-529L	MCF-7
34 (3-{3'-[iminooxy-O-((2-hydroxy-2-methyl)-propyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)	2.0 ± 0.5	2.0 ± 0.3
35 (2-{O-[2'-(2-oxo-(2H3H)indol-3-ylidene)-(2'H3'H)indol-3'-ylidene]-aminooxy}-acetic acid (sodium salt))	15.0 ± 0.8	20.5 ± 0.9
36 (3-{O-[2'-(2-oxo-(2H3H)indol-3-ylidene)-(2'H3'H)indol-3'-ylidene]-aminooxy}-propionic acid (sodium salt))	15.0 ± 0.8	16.0 ± 0.7
37 (4-{O-[2'-(2-oxo-(2H3H)indol-3-ylidene)-(2'H3'H)indol-3'-ylidene]-aminooxy}-butyric acid (sodium salt))	12.0 ± 1.0	14.0 ± 1.1
38 (5-{O-[2'-(2-oxo-(2H3H)indol-3-ylidene)-(2'H3'H)indol-3'-ylidene]-aminooxy}-pentanoic acid (sodium salt))	14.0 ± 0.8	14.0 ± 0.7
39 (3-{3'-(iminooxy-O-carbethoxy)-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)	4.5 ± 0.7	10.0 ± 0.9
40 (ethyl-2-{O-[2'-(2-oxo-(2H3H)indol-3-ylidene)-(2'H3'H)indol-3'-ylidene]-aminooxy}-acetate)	6.0 ± 0.4	10.0 ± 0.7
41 (3-{3'-[iminooxy-O-((N,N)-dimethyl-carbamoyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)	5.0 ± 0.6	7.5 ± 0.9
42 (2-{O-[2'-(2-oxo-(2H3H)indol-3-ylidene)-(2'H3'H)indol-3'-ylidene]-aminooxy}-acetamide)	2.0 ± 0.3	6.0 ± 0.5
43 (N,N-dimethyl-2-{O-[2'-(2-oxo-(2H3H)indol-3-ylidene)-(2'H3'H)indol-3'-ylidene]-aminooxy}-acetamide)	7.0 ± 1.1	9.2 ± 0.9
44 (2-{2-[O-(2'-(2-oxo-(2H3H)indol-3-ylidene)-(2'H3'H)indol-3'-ylidene)-aminooxy]-acetyl-amino}-acetic acid)	20.0 ± 0.8	18.0 ± 1.3

Table 4 (continued)

Example	SRB-Assay IC ₅₀ [μM]	
	LXFL-529L	MCF-7
45 (3-{3'-[iminooxy-O-(3-D-glucopyranosylpropyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)	19.0 ± 0.9	10.5 ± 0.6
46 (3-{3'-[iminooxy-O-(O ⁴ -α-D-glucopyranosyl-2-D-glucopyranosylethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)	24.0 ± 1.3	7.0 ± 0.9
47 (3-{3'-[iminooxy-O-(2-D-galactopyranosylethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)	17.0 ± 1.2	9.5 ± 0.9
48 (3-{3'-[iminooxy-O-(2-D-glucopyranosylethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)	21.0 ± 1.5	3.5 ± 0.6
49 (3-{3'-[iminooxy-O-(2-L-arabinopyranosylethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)	20.5 ± 1.4	5.0 ± 0.7
53 (3-{3'-[iminooxy-O-(2-(2-amino-2-deoxy-D-glucopyranosyl)-ethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)	15.0 ± 0.9	7.0 ± 0.7
54 (3-{3'-[iminooxy-O-(2-(2-amino-2-deoxy-D-galactopyranosyl)-ethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)	16.0 ± 1.1	9.0 ± 0.9
55 (3-{3'-[iminooxy-O-(N-(1-deoxy-glucitol)-2-amino-ethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)	3.0 ± 0.6	3.0 ± 0.8
56 (3-{3'-[iminooxy-O-(N-(2-deoxy-glucose)-2-amino-ethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)	12.0 ± 1.3	14.0 ± 1.2
57 (3-{3'-[iminooxy-O-(N-(2-deoxy-galactose)-2-amino-ethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)	23.0 ± 1.4	20.5 ± 1.6
58 (3-{3'-[iminooxy-O-((N,N)-dimethyl-2-amino-ethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)	5.0 ± 0.9	6.5 ± 1.1

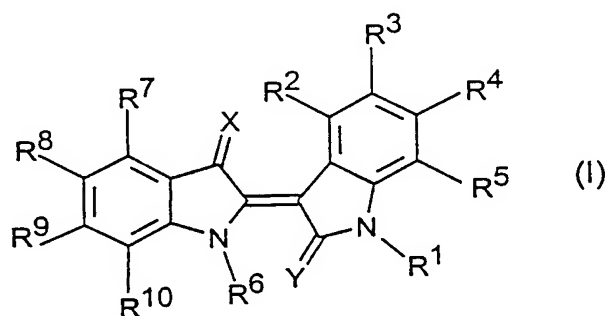
Table 4 (continued)

59 (3-{3'-[iminooxy-O-(N-hydroxyethyl-2-amino-ethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)	11.6 ± 0.8	13.6 ± 1.2
60 (3-{3'-[iminooxy-O-((N,N)-bis(hydroxyethyl)-2-amino-ethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)	3.0 ± 0.4	4.5 ± 0.5
61 (3-{3'-[iminooxy-O-((N,N)-bis(hydroxyethyl)-4-amino-butyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)	8.0 ± 0.3	12.4 ± 0.7
62 (3-{3'-[iminooxy-O-((N,N)-bis(hydroxyethyl)-10-amino-decyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)	14.0 ± 1.0	13.5 ± 1.3
63 (3-{3'-[iminooxy-O-(2-(piperazin-1-yl)-ethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)	8.0 ± 0.6	9.5 ± 0.8
64 (3-{3'-[iminooxy-O-(2-(morpholin-4-yl)-ethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)	10.0 ± 0.6	9.0 ± 1.2
65 (3-{3'-[iminooxy-O-(2-(4-methyl-piperazin-1-yl)-ethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)	6.5.0 ± 0.6	7.5 ± 0.8
66 (3-{3'-[iminooxy-O-(2-(2-amino-ethyl)-amino-ethyl)]-(2'H3'H)indol-2'-ylidene}-(2H3H)indol-2-one)	7.0 ± 0.6	8.0 ± 1.2
67 (3-{3'-[iminooxy-O-(2-hydroxy-ethyl)]-(2'H3'H)indol-2'-ylidene]-5-methyl-(2H3H)indol-2-one)	1.0 ± 0.5	1.5 ± 0.6
68 (3-{3'-[iminooxy-O-(2-D-glucopyranosylethyl)]-(2'H3'H)indol-2'-ylidene]-5-methyl-(2H3H)indol-2-one)	17.0 ± 0.8	2.5 ± 0.8
69 (3-{3'-[iminooxy-O-(N-(1-deoxy-glucitol)-2-amino-ethyl)]-(2'H3'H)indol-2'-ylidene]-5-methyl-(2H3H)indol-2-one)	3.5 ± 0.7	2.5 ± 0.9

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Claims

1. Cell membrane penetrating indigoid bisindole derivatives selected from indigo derivatives, isoindigo derivatives and indirubin derivatives, wherein the indigoid bisindole derivatives are compounds having the general formula (I)



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wherein:

X and Y are the same or different and represent an oxygen atom; a sulphur atom; a selenium atom; a tellurium atom; a group N-A-B-R¹⁴ in which A represents a single bond or an oxygen atom, -NH- or -NH-CO-, B represents a single bond or a group [(CD₂)_nZ]_m wherein D has the same meaning as R¹⁴ (see below) and Z is an oxygen atom or -NH-, n is 0 or an integer and m is an integer; and the group R¹⁴ represents a hydrogen atom, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can carry one or more hydroxy groups and/or amino groups and can be substituted by one or more carboxyl groups and/or phosphoryl groups, a substituted or unsubstituted aryl group which can comprise one or more heteroatoms, an aralkyl group, an acyl group, a glycoside selected from monosaccharides, disaccharides or oligosaccharides, or a group selected from the group consisting of sugars, amino acids, peptides or steroid hormones; or a hydrazone group N-NR¹⁵R¹⁶, wherein R¹⁵ and R¹⁶ can be the same or different and represent a hydrogen atom, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can be

substituted by one or more carboxyl groups and/or phosphoryl groups,, a substituted or unsubstituted aryl group which can comprise one or more heteroatoms, an aralkyl group, an acyl group, or a glycoside selected from monosaccharides, disaccharides or oligosaccharides, or a group selected from the group consisting of sugars, amino acids, peptides or steroid hormones;

R^2 , R^3 , R^4 , R^5 , R^7 , R^8 , R^9 and R^{10} can be the same or different and represent a hydrogen atom; a halogen atom; a hydroxy group; a nitroso group; a nitro group; an aryloxy group; an alkoxy group; a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups; a substituted or unsubstituted aryl group which can comprise one or more heteroatoms; a cycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatoms; an aralkyl group; a trifluoromethyl group; a -COM group; a -COOM group; a -CH₂COOM group, wherein M is hydrogen, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, or an aryl group which can comprise one or more heteroatoms and can be substituted with one or more halogen atoms, one or more alkyl groups or one or more alkoxy groups; a -NR¹¹R¹² group, wherein R¹¹ and R¹² can be the same or different and represent a hydrogen atom, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, a substituted or unsubstituted aryl group which can comprise one or more heteroatoms, or an acyl group, or R¹¹ and R¹² form together a ring having 2 to 6, optionally substituted, CH₂ groups; a benzyl group, wherein the benzene nucleus can comprise one or more heteroatoms; a hydroxylamino group; a phosphate group; a phosphonate group; a sulfate group; a sulfonamide group, wherein the nitrogen atom can be independently substituted by a hydrogen atom, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, a substituted or unsubstituted aryl group or wherein the nitrogen atom is part of a cycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatoms; an azo group -N=N-R¹³, in which R¹³ represents an

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aromatic system which can be substituted by one or more carboxyl groups and/or phosphoryl groups; or a O-glycoside or a N-glycoside, wherein the glycoside is selected from monosaccharides, disaccharides or oligosaccharides; or a group selected from the group consisting of sugars, amino acids, peptides or steroid hormones; or R¹ and R⁵, and R⁶ and R¹⁰, respectively, form independently from each other a ring together having 1 to 4, optionally substituted, CH₂ groups;

the groups R¹ and R⁶ are the same or different and represent a hydrogen atom; a halogen atom; a hydroxy group; a methylenehydroxy group; a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms; a cycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatoms; a substituted or unsubstituted aryl group which can comprise one or more heteroatoms; a mono-, di- or trialkylsilyl group having 1 to 6 carbon atoms independently of each other in each instance in the straight-chain or branched-chain alkyl group; a mono-, di- or triarylsilyl group with substituted or unsubstituted aryl groups independently of each other in each instance; an aralkyl group; a trifluoromethyl group; a -COM group; a -COOM group; a -CH₂COOM group, wherein M is hydrogen, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, or an aryl group which can comprise one or more heteroatoms and can be substituted with one or more halogen atoms, one or more alkyl groups or one or more alkoxy groups; a -NR¹⁷R¹⁸ group, wherein R¹⁷ and R¹⁸ can be the same or different and represent a hydrogen atom, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, a substituted or unsubstituted aryl group which can comprise one or more heteroatoms, or an acyl group; a methyleneamino group -CH₂-NR¹⁷R¹⁸, wherein R¹⁷ and R¹⁸ have the above definitions; a benzyl group, wherein the benzene nucleus can comprise one or more heteroatoms; a methylenecycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatoms; a physiological amino acid residue bound to the nitrogen as an amide; an O-glycoside or a N-glycoside, wherein the glycoside is selected from monosaccharide, disaccharides or oligosaccharides; or a

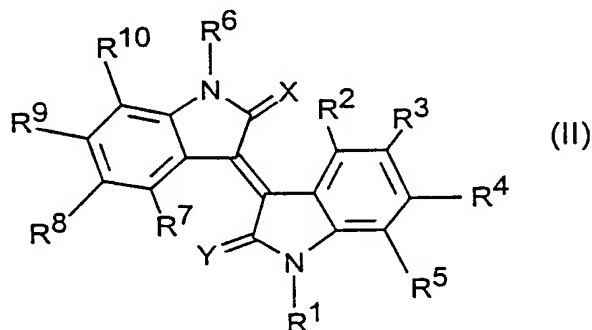
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group selected from the group consisting of sugars, amino acids, peptides or steroid hormones; or a methylene sulfonate group.

2. Indigoid bisindole derivative according to claim 1, wherein one or more ring atoms of the benzene nuclei of the compound having the general formula (I) are replaced by nitrogen atoms.

3. Indigoid bisindole derivative according to claims 1 or 2, wherein the compound having the general formula (I) is bound to a polyethyleneglycol-ester or a polyethyleneglycolether.

4. Indigoid bisindole derivative according to claim 1, wherein the isoindigo derivative is a compound having the general formula (II)



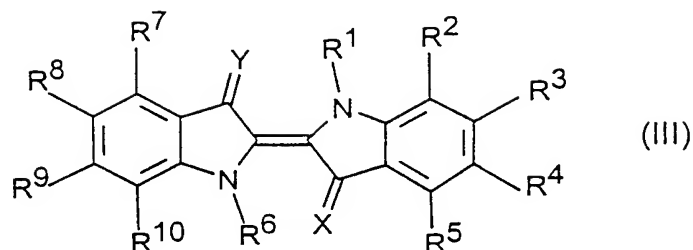
wherein R¹ to R¹⁰ and X and Y have the meanings as defined in claim 1.

5. Indigoid bisindole derivative according to claim 4, wherein one or more ring atoms of the benzene nuclei of the compound having the general formula (II) are replaced by nitrogen atoms.

6. Indigoid bisindole derivative according to claim 4 or 5, wherein the compound having the general formula (II) is bound to a polyethyleneglycol-ester or a polyethyleneglycolether.

7. Indigoid bisindole derivative according to claim 1, wherein the indigo derivative is a compound having the general formula (III)

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wherein R¹ to R¹⁰ and X and Y have the meanings as defined in claim 1.

8. Indigoid bisindole derivative according to claim 7, wherein one or more ring atoms of the benzene nuclei of the compound having the general formula (III) are replaced by nitrogen atoms.

9. Indigoid bisindole derivative according to claim 7 or 8, wherein the compound having the general formula (III) is bound to a polyethyleneglycol-ester or a polyethyleneglycolether.

10. Indigoid bisindole derivative according to any one of the preceding claims, wherein the indigoid bisindole derivative is in the form of a physiologically acceptable salt.

11. Pharmaceutical formulation comprising at least one of the indigoid bisindole compounds according to any one of claims 1 to 10.

12. Use of the pharmaceutical formulation according to claim 11 for the manufacture of a medicament for the treatment of human solid tumors.

INTERNATIONAL SEARCH REPORT

Intern: al Application No

PCT/EP 00/03285

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D209/34 C07D209/40 A61K31/404 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, PAJ, BEILSTEIN Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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P, X	WO 99 62503 A (EISENBRAND GERHARD ;CNRS CENTRE NATIONAL DE RECH S (FR)) 9 December 1999 (1999-12-09) the whole document	1-12
	-/-	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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